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PATENT
Attorney Docket No.: 020547-003700US
Client Reference No.: 010110.00

Amendments to the Claims:

A listing of the pending claims is provided below for the convenience of the Office:

Listing of Claims:

1. (*Currently amended*) A method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order, said method comprising:

a) providing at least three different DNA vectors ~~molecules~~, each comprising a DNA segment, wherein each segment is adjacent to one or two other segments in the ligation product, wherein each segment comprises a first region having sequence identity with a first adjacent DNA segment and a second region having sequence identity with a second adjacent DNA segment, if present, and wherein each vector comprises a selectable marker;

b) cleaving each DNA molecule to produce a DNA segment with one or two ligatable ends, each ligatable end comprising at least a portion of the region having sequence identity with an adjacent DNA segment; wherein at least one segment comprises two ligatable ends after cleavage,

c) simultaneously ligating each segment to the adjacent segment or segments; and

d) selecting a ligation product comprising sequences from each of said DNA segments in a predetermined order, wherein said selection is based on a selectable marker of one of said three DNA vectors.

2. *(Original)* A method for ligation of a plurality of DNA segments to obtain a ligation product that comprises sequences from each of said DNA segments in a predetermined order, said method comprising:

a) providing a Type 1 DNA molecule, a Type 2 DNA molecule and at least one Type 3 DNA molecule, each comprising a DNA segment that is adjacent to one or two other segments in the final ligation product, wherein

i) said Type 1 DNA molecule comprises a first DNA segment, a first selectable marker, a first counter-selectable marker, a first cleavage site, and a second cleavage

site, wherein cleavage of said second cleavage site produces a single-strand overhang in said first DNA segment which is ligatable to a single-strand overhang of an adjacent segment;

ii) said Type 2 DNA molecule comprises a second DNA segment, a second selectable marker, a second counter-selectable marker, a third cleavage site, and a fourth cleavage site, wherein cleavage of said fourth cleavage site produces a single-strand overhang in said second DNA segment which is ligatable to a single-strand overhang of an adjacent segment;

iii) each said Type 3 DNA molecule comprises a DNA segment, a third counterselectable marker, a 5-prime cleavage site and a 3-prime cleavage site, wherein said 5-prime cleavage site, upon cleavage, produce a single-strand overhang in the segment that is ligatable to a single-strand overhangs of an adjacent segment, and said 3-prime cleavage site, upon cleavage, produce a single-strand overhang in the segment that is ligatable to a single-strand overhangs of a different adjacent segment;

wherein said first and second selectable markers are different;

wherein said first, second and third counter-selectable markers are independently selected and are the same or different;

wherein said first and third cleavage sites the same or are compatible;

wherein said second and fourth cleavage sites are independently selected and are the same or are different; and,

wherein each 5-prime and 3-prime cleavage site is independently selected in each Type 3 DNA molecule and are the same or are different;

b) cleaving each DNA molecule at the first, second, third, and fourth cleavage sites, at the 5-prime cleavage site(s) and at the 3-prime cleavage site(s); and

c) ligating the resulting fragments to each other thereby producing a ligation product that comprises sequences from each of said DNA segments in a predetermined order.

3. *(Original)* The method of claim 2 further comprising the steps

d) transforming cells with ligation products produced in step (c); and

e) selecting transformants that express said first and second selectable markers and do not express said first, second, or third counter-selectable marker.

4. *(Original)* The method of claim 3 further comprising the step:

f) isolating the ligation product comprising sequences from each of said DNA segments in a predetermined order from the transformants or their progeny.

5. *(Original)* The method of claim 2 wherein said first and second selectable markers are genes conferring drug resistance.

6. (*Currently amended*) The method of claim 2 wherein said first, second and third counter-selectable markers are selected from the group consisting of *ccdB* (anti-DNA gyrase protein), *sacB* (sucrose sensitivity), *araB* (ribulose sensitivity), *tetAR* (tetracycline resistance/fusaric acid hypersensitivity)[,] .

7. *(Original)* The method of claim 2 wherein

a) said first and third cleavage sites are the same;

b) said second and fourth cleavage sites are the same;

c) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 3- prime cleavage site of the same Type 3 DNA molecule; and/or

d) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 5- prime cleavage site of a different Type 3 DNA molecule.

8. *(Original)* The method of claim 2 wherein

a) said first and third cleavage sites are sites cleaved by a Type IIS restriction enzyme;

b) said second and fourth cleavage sites are sites cleaved by a Type IIS restriction enzyme; and/or

c) said 5-prime and 3-prime cleavage sites of at least one Type 3 DNA molecule are sites cleaved by a Type IIS restriction enzyme.

9. *(Original* The method of claim 2 wherein the first, second, third, fourth, 5-prime and 3-prime cleavage sites are sites cleaved by a Type IIS restriction enzyme.

10. *(Original)* The method of claim 2 wherein the first, second, third, fourth, 5-prime and 3-prime cleavage sites are sites cleaved by the same Type IIS restriction enzyme.

11. *(Original)* The method of claim 2 wherein the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polypeptide segment of a polyketide synthase.

12. *(Original)* The method of claim 2 wherein the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polyketide synthase domain.

13. *(Original)* The method of claim 2 wherein the DNA molecules cleaved in step (b) are cleaved in the same container.

14. *(Original)* A composition comprising:

i) a Type 1 DNA molecule, said DNA molecule comprising a first DNA segment, a first selectable marker, a first counter-selectable marker, a first cleavage site, and a second cleavage site; wherein cleavage of said second cleavage site produces a single-strand overhang in said first segment which is ligatable to a single-strand overhang of an adjacent segment;

ii) a Type 2 DNA molecule, said DNA molecule comprising a second DNA segment, a second selectable marker, a second counter-selectable marker, a third cleavage site, and a fourth cleavage site wherein cleavage of said fourth cleavage site produces a single-strand overhang in said first segment which is ligatable to a single-strand overhang of an adjacent segment;

iii) at least one Type 3 DNA molecule, said DNA molecule comprising a DNA segment, a third counter-selectable marker, a 5-prime cleavage site and a 3-prime cleavage site, wherein said 5-prime and 3-prime cleavage sites, upon cleavage, produce single-strand overhangs in the segment that are ligatable to a single-strand overhangs of each of two adjacent segments;

wherein said first and second selectable markers are different;

wherein said first, second and third counter-selectable markers are independently selected and are the same or different;

wherein said first and third cleavage sites the same or are compatible;

wherein said second and fourth cleavage sites are independently selected and are the same or are different; and,

wherein each 5-prime and 3-prime cleavage site is independently selected.

15. *(Original)* The composition of Claim 14 comprising at least two Type 3 DNA molecules.

16. *(Original)* The composition of Claim 14 comprising an endonuclease that cleaves at the first, second, third, or fourth cleavage sites or at one or more 5-prime or 3-prime cleavage sites.

17. *(Original)* The composition of Claim 16 wherein the endonuclease cleaves at the first, second, third, and fourth cleavage sites and at one or more 5-prime or 3-prime cleavage sites.

18. *(Original)* The composition of Claim 16 that contains at least two Type 3 DNA molecules comprising 5-prime or 3-prime cleavage sites and wherein the endonuclease cleaves at the third and fourth cleavage sites and at the 5-prime and 3-prime cleavage sites of said Type 3 DNA molecules.

19. *(Original)* A composition comprising the products resulting from cleavage of the Type 1, Type 2 and Type 3 DNAs of Claim 14 at the first, second, third, fourth, 5-prime and 3-prime cleavage sites.

20. (*Original*) The composition of Claim 19 additionally containing DNA ligase.

21. (*withdrawn*) A cloning vector comprising, in the order shown,

a) SIS - CSM- R - USM or SIS - USM - R - CSM; or

b) SIS-CSM-SM

where SIS is a synthon insertion site, CSM is a counter-selectable marker; SM and USM are selectable markers, and R is an endonuclease cleavage site.

22. (*withdrawn*) The vector of claim 20 wherein the SIS comprises - N_1-R_2-N_2 - where N_1 and N_2 are recognition sites for nicking enzymes, and may be the same or different, and R_2 is a recognition site for an endonuclease.

23. (*withdrawn*) The vector of claim 20 wherein SM and USM are genes conferring drug resistance.

24. (*withdrawn*) A vector comprising, in the order shown:

a) R_1 - Sy - $2S_1$ - CSM- R_2-USM

b) $2S_2$-Sy-R_3 -USM-R_4-CSM;or

C) $2S_3$ - Sy - $2S_4$ - CSM - SM

where $2S_1$, $2S_2$, $2S_3$ and $2S_4$ are recognition sites for Type IIS restriction enzymes, which may be the same or different,

Sy is a synthon coding region

R_1 and R_3 are endonuclease cleavage sites

R_2 and R_4 are endonuclease cleavage sites that, upon cleavage, result in compatible ends

CSM is a counter-selectable marker; and,

USM and SM are selectable markers.

25. (*withdrawn*) A composition comprising the vector of claim 24 and an endonuclease that cleaves at one or more of R_1, R_2, R_3, R_4, $2S_1$, $2S_2$, $2S_3$, or $2S_4$.

26. (*withdrawn*) A composition comprising each of the

a) a vector of the formula R_1 - Sy - $2S_1$ - CSM- R_2-USM

b) a vector of the formula $2S_2$ - Sy - R_3 - USM - R_4 - CSM; and

c) a vector of the formula $2S_3$ - Sy - $2S_4$ - CSM - SM

wherein $2S_1$, $2S_2$, $2S_3$ and $2S_4$ are recognition sites for Type IIS restriction enzymes, which may be the same or different,

each Sy is a different synthon coding region,

R_1 and R_3 are endonuclease cleavage sites and are the same or are different,

R_2 and R_4 are endonuclease cleavage sites that, upon cleavage, result in compatible ends,

$2S_1$, $2S_2$, $2S_3$, and $2S_4$ are endonuclease cleavage sites and are the same or are different,

CSM is a counter-selectable marker; and,

USM and SM are selectable markers.

27. (*withdrawn*) The composition of claim 26 that comprises 2, 3, 4, 5, or 6 vectors of the formula $2S_3$ - Sy - $2S_4$ - CSM - SM, each with a different Sy.

28. (*withdrawn*) The composition of claim 26 further comprising an endonuclease that cleaves at one or more of R_1, R_2, R_3, R_4, $2S_1$, $2S_2$, $2S_3$, and $2S_4$.

29. (*withdrawn*) A synthetic gene encoding a domain of a polyketide synthase that corresponds to a domain of the polyketide synthase encoded by a naturally occurring gene, wherein the domain-encoding sequence of the synthetic gene is different from the domain-encoding sequence of the naturally occurring gene, wherein

a) said domain-encoding sequence of said synthetic gene is less than about 80% identical to said domain-encoding sequence of said naturally occurring gene, and/or

b) said domain-encoding sequence of said synthetic gene comprises at least one unique restriction site that is not present or is not unique in the domain-encoding sequence of said naturally occurring gene, and/or

c) said domain-encoding sequence of said synthetic gene is free from at least one restriction site that is present in the domain-encoding sequence of said naturally occurring gene, and/or

d) the codon usage distribution in said domain-encoding sequence is substantially different from that of the naturally occurring gene.

30. (*withdrawn*) A method for synthesis of a gene encoding a polypeptide segment comprising

a) designing a synthetic gene encoding the polypeptide segment;

b) designing component oligonucleotides for synthesis of the gene;

c) synthesizing the gene by generating synthons from said component oligonucleotides and stitching two or more synthons together.

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Conclusion

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

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Respectfully submitted,

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